

Listing of Claims

1-25 (Cancelled)

26. (Previously Presented) A method of measuring the angiogenic or antiangiogenic activity of a test molecule comprising:

- (a) obtaining an embryonated fowl egg,
- (b) creating a window in the shell of the fowl egg, such that the chorioallantoic membrane (CAM) is exposed,
- (c) providing to a test region of interest on the CAM a substrate,
- (d) administering to a vessel located in the CAM a test molecule,
- (e) administering to a vessel located in the CAM a fluorescent-labeled particle, such that the fluorescent-labeled particle travels through each vessel contained in the test region of interest,
- (f) removing the substrate and the test region of interest from the fowl egg,
- (g) capturing a three-dimensional image of the test region of interest, wherein the three-dimensional image comprises a plurality of pixels, such that a fluorescent vascular density (FVD) value can be assigned to the test region of interest, and
- (h) comparing the FVD value of the test region of interest with the FVD value of a control region of interest that was prepared in the same manner as the test region of interest but without the administration of a test molecule or with the administration of a control molecule, such that the angiogenic or antiangiogenic activity of the test molecule is measured,

wherein a lower FVD value of the test region of interest as compared to the FVD value of the control region of interest is indicative of the test molecule being useful as an inhibitor of angiogenesis, and

wherein a higher FVD value of the test region of interest as compared to the FVD value of the control region of interest is indicative of the test molecule being useful as a stimulator of angiogenesis.

27. (Previously Presented) The method of claim 26, wherein the embryonated fowl egg is a chicken egg.
28. (Previously Presented) The method of claim 26, wherein the substrate is selected from the group consisting of glass, plastic, nylon, silicon, polytetrafluoroethylene, Matrigel, collagen, fibrinogen, agarose, methylcellulose, and filter paper.
29. (Previously Presented) The method of claim 28, wherein the substrate comprises a stimulator of angiogenesis, such that angiogenesis is stimulated in the region of interest.
30. (Previously Presented) The method of claim 29, wherein the stimulator of angiogenesis is selected from the group consisting of a synthetic molecule, a nucleic acid sequence encoding a stimulator of angiogenesis, a polypeptide that can stimulate angiogenesis, a biological tissue containing a stimulator of angiogenesis, and a cell containing a stimulator of angiogenesis.
31. (Previously Presented) The method of claim 28, wherein the substrate comprises an inhibitor of angiogenesis, such that angiogenesis is inhibited in the region of interest.
32. (Previously Presented) The method of claim 31, wherein the inhibitor of angiogenesis is selected from the group consisting of a synthetic molecule, a nucleic acid sequence encoding an inhibitor of angiogenesis, a polypeptide that can inhibit angiogenesis, a biological tissue containing an inhibitor of angiogenesis, and a cell containing an inhibitor of angiogenesis.
33. (Previously Presented) The method of claim 26, wherein the test molecule and the fluorescent-labeled particle are administered to different vessels in the CAM.
34. (Previously Presented) The method of claim 33, wherein each vessel is cannulated prior to administration of the test molecule and the fluorescent-labeled particle.
35. (Previously Presented) The method of claim 34, wherein the fluorescent-labeled particle is selected from the group consisting of a fluorescent-labeled carbohydrate, a

fluorescent-labeled protein, polypeptide, or peptide, and a fluorescent-labeled synthetic polymer.

36. (Previously Presented) The method of claim 35, wherein the fluorescent-labeled particle is labeled with a fluorescent moiety selected from the group consisting of fluorescein, green fluorescent protein, yellow fluorescent protein, Lucifer yellow, rhodamine, cyanine based compounds, C6-NBD, DIO-Cn-(3), BODIPY-FL, eosin, propidium iodide, Dil-Cn-(3), Cy3, Texas Red, Dil-Cn-(5), allophycocyanin, and Cy5.

37. (Previously Presented) The method of claim 36, wherein the fluorescent-labeled particle is labeled with a fluorescent moiety that can be excited by a laser and the three-dimensional image is captured by laser confocal microscopy.

38. (Previously Presented) The method of claim 37, wherein the three-dimensional image is formed by taking multiple cross-sectional images along a three-dimensional z-axis of the region of interest.

39. (Previously Presented) The method of claim 38, wherein the three-dimensional image is analyzed by computer analysis of the relative fluorescent brightness of each pixel contained in the three-dimensional image to determine an FVD value for each vessel contained in each cross-sectional image.

40. (Previously Presented) The method of claim 39, wherein the FVD value of the region of interest is calculated by:

- (a) assigning a brightness value ranging from 0 to 255 to each pixel,
- (b) applying a thresholding algorithm to the image,
- (c) selecting a low threshold such that any pixel with less brightness than the low threshold is deleted,
- (d) optionally, selecting a high threshold such that any pixel with greater brightness than the high threshold is deleted,
- (e) summing the remaining pixels to determine the FVD value of all of the vessels in each cross-sectional image of the region of interest, and

(f) averaging the FVD values of at least two of the brightest cross-sectional images to determine the FVD value of the region of interest.

41. (Currently Amended) A method of measuring the angiogenic or ~~antiangiogenic~~ antiangiogenic activity of a test molecule comprising:

- (a) obtaining an embryonated fowl egg,
- (b) creating a window in the shell of the fowl egg, such that the CAM is exposed,
- (c) providing to a test region of interest on the CAM a substrate,
- (d) administering to a vessel located in the CAM a test molecule,
- (e) administering to a vessel located in the CAM an agent to measure metabolic activity,
- (f) removing the substrate and the test region of interest from the fowl egg,
- (g) measuring the spectrophotometric absorbance value of the test region of interest, and
- (h) comparing the spectrophotometric absorbance value of the test region of interest with the spectrophotometric absorbance value of a control region of interest that was prepared in the same manner as the test region of interest but without the administration of a test molecule or with the administration of a control molecule, such that the angiogenic or antiangiogenic activity of the test molecule is measured,

wherein a lower spectrophotometric absorbance value of the test region of interest as compared to the spectrophotometric absorbance value of the control region of interest is indicative of the test molecule being useful as an inhibitor of angiogenesis, and

wherein a higher spectrophotometric absorbance value of the test region of interest as compared to the spectrophotometric absorbance value of the control region of interest is indicative of the test molecule being useful as a stimulator of angiogenesis.

42. (Previously Presented) The method of claim 41, wherein the agent is XTT, MTT, or WST-1.

43. (Previously Presented) The method of claim 41, wherein the embryonated fowl egg is a chicken egg.

44. (Previously Presented) The method of claim 42, wherein the substrate is selected from the group consisting of glass, plastic, nylon, silicon, polytetrafluoroethylene, Matrigel, collagen, fibrinogen, agarose, methylcellulose, and filter paper.
45. (Previously Presented) The method of claim 44, wherein the substrate comprises a stimulator of angiogenesis, such that angiogenesis is stimulated in the region of interest.
46. (Previously Presented) The method of claim 44, wherein the stimulator of angiogenesis is selected from the group consisting of a synthetic molecule, a nucleic acid sequence encoding a stimulator of angiogenesis, a polypeptide that can stimulate angiogenesis, a biological tissue containing a stimulator of angiogenesis, and a cell containing a stimulator of angiogenesis.
47. (Previously Presented) The method of claim 44, wherein the substrate comprises an inhibitor of angiogenesis, such that angiogenesis is inhibited in the region of interest.
48. (Previously Presented) The method of claim 47, wherein the inhibitor of angiogenesis is selected from the group consisting of a synthetic molecule, a nucleic acid sequence encoding an inhibitor of angiogenesis, a polypeptide that can inhibit angiogenesis, a biological tissue containing an inhibitor of angiogenesis, and a cell containing an inhibitor of angiogenesis.
49. (Previously Presented) The method of claim 46, wherein the test molecule and the agent to measure metabolic activity are administered to different vessels in the CAM.
50. (Previously Presented) The method of claim 49, wherein each vessel is cannulated prior to administration of the test molecule and the agent to measure metabolic activity.